

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Schneider et al.

Confirmation No. 8951

Serial No.: 10/630,375

Filing Date: July 29, 2003

For: **RECONSTITUTABLE FORMULATION AND AQUEOUS
SUSPENSION OF GAS-FILLED MICROVESICLES FOR DIAGNOSTIC IMAGING**

Examiner: Nabila G. EBRAHIM.

Group Art Unit: 1618

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Sir:

RULE 132 DECLARATION OF MICHEL SCHNEIDER, PH.D.

I, Michel Schneider, Ph.D., declare as follows:

1. I am a citizen of Switzerland, and currently reside in 34 route d'Annecy, Troinex (Switzerland).
2. I received a Master of Science degree in chemistry in 1965 from University of Nancy (France). I received a Ph.D. in Physical Chemistry in 1969 from Laval University (Canada).
3. For over 21 years, I have been engaged in the research and development of diagnostic imaging agents, including ultrasound contrast agents and dried formulations used to prepare such contrast agents. Since 1989, I have been, and currently am, employed by Bracco Research Geneva (formerly Sintetica), 31 route de la Galaise, Plan-les-Ouates, Switzerland ("Bracco Research") as Director until January 2010, then as Senior Scientific Advisor, with duties relating

U.S.S.N. 10/630,375

to overseeing research and development of diagnostic imaging agents, including ultrasound contrast agents and formulations used to prepare such agents.

4. I am also an inventor and/or co-inventor of numerous patents owned by Bracco, including various United States, European, and other patents worldwide relating to constructs for diagnostic imaging and therapy, and methods of making the same. I am a co-inventor of U.S. Patent Application No. 10/630,375.

5. I have reviewed the above referenced U.S.S.N. 10/630,375, including the latest pending claims, and am familiar with the subject matter disclosed and claimed therein.

6. I have reviewed the Office Action mailed December 29, 2010 ("Office Action"), and the references cited therein (Schneider et al EP 0554213 ("Schneider" or the "Schneider Reference") and Van Liew et al, J. Appl Physiol. 82:2045-2053 (1997) ("Van Liew" or the "Van Liew Reference")) and make this declaration in support of the concurrently filed *Amendment After Final and Response To Office Action*. I am a co-inventor of the Schneider reference.

The Claimed Invention

7. I have reviewed the currently pending claims, which are directed to a sealed container comprising: 1) a dried material comprising at least one film forming surfactant; and 2) a gas at a pressure lower than atmospheric pressure.

8. These containers contain a dry formulation which is a contrast agent precursor. The dry formulation is stored in the container in contact with gas at a pressure below atmospheric pressure. When a contrast agent is desired the dry formulation is dissolved in an aqueous liquid carrier to produce a suspension of gas-filled microvesicles which may be used as a contrast agent. This process is referred to as reconstitution in the specification. See e.g. paragraphs 0012-0013 in the specification.

9. Once the aqueous carrier liquid is added to the container, the gas in the container, the microvesicles in the suspension and the surrounding medium are all necessarily at atmospheric pressure.

10. Due to the fact that a gas at reduced pressure is introduced into the sealed container, lower amounts of gas can be employed, as compared to a vial with the same volume containing the gas at atmospheric pressure, as mentioned on paragraphs 45-48 of the US publication of the instant application (US 2005/0025710), hereinafter ("the application"). The reduced pressure into the vial further facilitates the introduction of the diluent for the reconstitution of the lyophilizate (see par. 44 of the application)

11. We unexpectedly found that, notwithstanding the reduced amount of gas in the vial, the microvesicles obtained from the reconstitution of the lyophilizate in said container show comparable stability characteristics with respect to microvesicles obtained by reconstituting a lyophilizate under atmospheric pressure, as illustrated in example 14 of the application (see in particular tables 2 to 5).

12. We also found that suspensions of microvesicles produced from the claimed containers including a gas at lower than atmospheric pressure unexpectedly had microvesicles of consistently reproducible size and had a reduced percentage of large bubbles, independent of the amount of agitational energy applied during reconstitution. Specifically, dry formulations stored in containers of the invention permit the production of microvesicle suspensions with substantially fewer microvesicles larger than 10 μm . For instance, dry formulations in containers with gas at 500 mbar can yield microvesicle suspensions with less than 50% the number of microvesicles over 8 μm than dry formulations at atmospheric pressure. This is reported in, for example, paragraphs 0038 and 0126 of the application and in Examples 9-11.

The Schneider Reference

13. I have reviewed EP 0554213 to Schneider et al. I am a co-inventor of this patent application.

14. All dry formulations in the Schneider reference are stored under gas at atmospheric pressure. See e.g. Schneider p. 4, line 36-40, emphasis added ("Hence, in one preferred case where microbubbles are to be formed from an aqueous phase and dry laminarized phospholipids, e.g. powders of dehydrated lyophilized liposomes plus stabilizers, which powders are to be subsequently dispersed under agitation in a liquid aqueous carrier phase, it is advantageous to store this dry powder under an atmosphere of a gas selected according to the invention.") There is no suggestion to vary the pressure of the gas used in dry formulations, never mind a suggestion of the advantages of storing compositions under a gas at a pressure lower than atmospheric as in the claimed invention.

15. The only disclosure in Schneider of use gas at pressure lower than atmospheric is as a transient and intermediate step in a process to replace a first gas with a second, desired gas, which is at ambient, not reduced pressure:

For instance, the vesicle suspensions, or preferably precursors thereof (precursors here may mean the materials the microvesicle envelopes are made of, or the materials which, upon agitation with an aqueous carrier liquid, will generate or develop the formation of microbubbles in this liquid), can be exposed to reduced pressure to evacuate the gas to be removed and then the ambient pressure is restored with the desired gas for substitution.

Schneider, p. 4, lines 29-33 (emphasis added). During this replacement process, the container is not sealed as required by the instant claims. Indeed, Schneider makes clear that once the container is sealed, the dried material is at ambient pressure "under an atmosphere of a gas selected according to the invention." Schneider, p. 4, lines 39-40.

The Van Liew Reference

16. I have reviewed the Van Liew reference. Van Liew is directed to the properties of aqueous suspensions of microbubbles and does not discuss dried formulations used to prepare such

suspensions. It does not discuss the storage of dry formulations or sealed containers used to store such formulations. Van Liew does not suggest use of gas at pressures below atmospheric in dry formulations or in the containers used to store such formulations. It neither teaches nor suggests the advantages of the claimed invention discussed above.

17. While van Liew does not discuss the claimed dry formulations including gas at pressures lower than atmospheric, it does discuss "negative pressure" in the context of suspensions of stabilized microbubbles: "The crucial aspect of a structural stabilizer is that it must produce a **negative pressure inside the bubble** to counter the tendency for outward diffusion of the gases inside, especially to counter the strong positive internal pressure due to surface tension when the bubbles are small". Van Liew p. 2045 col. 2, lines 24 et seq. However, as discussed above, the claims are directed to a sealed container which includes dried material and a gas at pressure lower than atmospheric.

18. The phrase "negative pressure" in Van Liew does not refer to an "absolute" negative pressure (in the sense that the pressure inside the bubble is lower than the pressure in the surrounding medium, i.e. atmospheric pressure), but rather to a pressure which counters (i.e. having an opposite direction) the internal overpressure (with respect to the surrounding pressure) caused by surface tension at the gas-liquid interface.

19. This becomes clear by reading the subsequent mathematical explanation across page 2046. The presence of the stabilizer is intended to act as a counterpressure (P_r) against the hydrostatic pressure (P_γ) exerted by the surface tension. See col. 2 first paragraph ("A stabilization mechanism exerts a counterpressure (P_r) against the tendency of surface tension and other forces to cause outward diffusion of the bubble's gaseous contents.") . Without the stabilizing layer, the gas contained in the bubble will be forced to diffuse outwardly, because of this hydrostatic pressure. The fact that the counterpressure exerted by the stabilizer is referred to by the author as a "negative

pressure" (opposing the "positive" hydrostatic pressure) is only a matter of mathematical convention, to indicate an opposite direction from the hydrostatic pressure, as inferable from equation 3 at the bottom of page 2046, col. 2. In this equation, the hydrostatic pressure " P_y " appears as a positive value, while the counterpressure " P_r ", generated by the stabilizer, is indicated as a negative value. Thus, the "negative" pressure generated by the stabilizer balances the hydrostatic pressure on the bubble, to avoid diffusion of the gas in the liquid – it does not mean that the pressure of the gas inside the bubble is lower than the pressure in the surrounding medium. In fact, the bubbles suspension reach an equilibrium state where the pressure inside the bubble, the surrounding medium and the air above are all at atmospheric pressure.

I HEREBY DECLARE that all statements made of my own knowledge are true, and all statements made on information and belief are believed to be true. I make this declaration understanding that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 17th, 2010

Respectfully submitted,

By:



Michel Schneider, Ph.D.